

CHROMOSOME ASSOCIATIONS IN ONION ROOT TIP NUCLEI

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ABSTRACT

Fluorescence analysis of *Allium cepa* nuclei has provided evidence for an ordered arrangement of chromosomes during interphase. In addition it is shown to be heterochromatin that is involved in the end-to-end association of chromosomes that has been observed during prophase, and it is therefore suggested that one function of this type of chromatin may be the maintenance of an ordered chromosome arrangement between cell divisions.

UITTREKSEL

CHROMOSOOM ASSOSIASIES IN DIE UI WORTELPUNT KERNE

Fluoresserende analyses van *Allium cepa*-kerne lewer bewyse vir die ordelike rangskikking van chromosome gedurende interfase. Daar word ook getoon dat heterochromatien betrokke is by die kop aan kop assosiasie van chromosome wat gedurende die profase waargeneem is, en word dit voorgestel dat een funksie van die tipe chromatin die behoud van 'n ge-ordende chromosoomrangskikking tussen seldelings is.

INTRODUCTION

It has been known for some time that certain regions of chromosome differ from the remainder in their condensation cycle, being represented variously as bands in metaphase chromosomes and as condensed "chromocentres" in interphase nuclei. Regions of chromosome showing such behaviour are termed "allo-cyclic" or "heterochromatic". During recent years great attention has been devoted to these regions, following the discovery by Caspersson *et al.* (1968) that they show differential fluorescence after treatment with certain dyes and irradiation by ultraviolet light, and the observations of Pardue and Gall (1970) that they may be differentially stained with Giemsa following a denaturation-reannealing treatment.

In this investigation, following the techniques of Vosa and Marchi (1972) and Vosa (1973), a combined denaturation-reannealing-fluorescence method was used to study chromosomal arrangements in onion nuclei during interphase and early prophase.

MATERIAL AND METHODS

Root tips of *Allium cepa* var. "Ailsa Craig" were pretreated in 0.1% colchicine for 4-5 hours at room temperature, fixed in 3:1 ethanol : glacial acetic acid

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Accepted for publication 30th July, 1976.

overnight, and hydrolysed in 0.2N HCl at 60° C for 2½ minutes. This was followed by squashing in 45% acetic acid under an albumenised coverslip, the coverslip then being floated off in absolute alcohol, air dried, immersed in saturated barium hydroxide for 5 minutes at room temperature, and incubated in 2 × saline sodium citrate for 30 minutes at 60° C. The coverslip was then washed, air dried, stained for 5 minutes in a benzimidazole derivative (Hoechst 33258-0.1% in absolute alcohol) and observed using a Zeiss ultraphot microscope with an exciter filter BG12 and barrier filter 50.

RESULTS

While other studies, employing Giemsa have indicated the presence of both terminal and centromeric heterochromatin in this species (Mogford, unpublished data; Vosa, in prep.), the present method demonstrates principally the terminal heterochromatin. Terminal heterochromatin was present on all the chromosomes, and, in particular, constituted the highly fluorescent satellites possessed by one chromosome pair (Fig. 1).

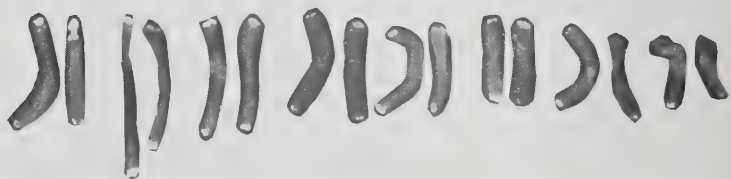


FIG. 1.

The chromosome complement of *Allium cepa*, following the fluorescence technique described in text. Note the highly fluorescent satellites of the first chromosome pair. X1250.

Examination of interphase nuclei indicates that the terminal heterochromatic regions are largely grouped on one side of the nucleus (Fig. 2). Examination of a given cell by both fluorescence and phase contrast indicates that the fluorescent regions do not correspond to any phase-retarding bodies, while examination of early prophase nuclei indicates that in several instances chromosomes are attached terminally at this stage.

DISCUSSION

The observation that the regions of terminal heterochromatin do not correspond to those of any phase-retarding bodies implies that the presence of denser regions in resting nuclei may not be considered a definitive criterion for the presence of heterochromatin.

The grouping of terminal regions at one side of the interphase nucleus is consistent with the expected pattern of chromosome alignment at the previous telophase. Other evidence of an ordered pattern of chromosomes in interphase nuclei exists, the principal being the evidence for an ordered arrangement of chromosomes in the head of the human sperm (Barlow and Vosa, 1970).

The observation that the terminal regions of the chromosomes are heterochromatic and that it is these regions which show fusion at early prophase, throws new light on the striking observation of Wagenaar (1969) that at this stage the chromosomes in this species may be present as a continuous chain, with presumably the homologous chromosomes joined end-to-end. It is clearly a possibility that the formation of terminal attachments, which were interpreted by Wagenaar as being concerned with the maintenance of chromosome arrangement during interphase, may be one of the functions of heterochromatin.

It should be pointed out that the heterochromatic regions revealed by the above technique are thought to contain highly repetitive DNA, which by virtue of such repetition alone would tend to reanneal together during the incubation in buffer following barium treatment. However, Wagenaar's observations on terminal associations in acetic-orcein and Feulgen squashes would imply that such fusions are not solely an artefact of the denaturation-reannealing treatment.

It should also be pointed out that because of the differences in length of non-homologous chromosomes, it would be expected from the telophase arrangement alone that it would be homologous chromosomes which attach preferentially to each other. This, however, solely suggests a mechanism for the formation of such terminal attachments and does not necessarily deny their possible adaptive significance along the lines suggested above.

ACKNOWLEDGMENTS

May I thank Professor F. R. Whatley for the provision of facilities at the Oxford Botany School, and Dr. C. G. Vosa for invaluable advice. The financial assistance of the S.R.C. is also gratefully acknowledged.

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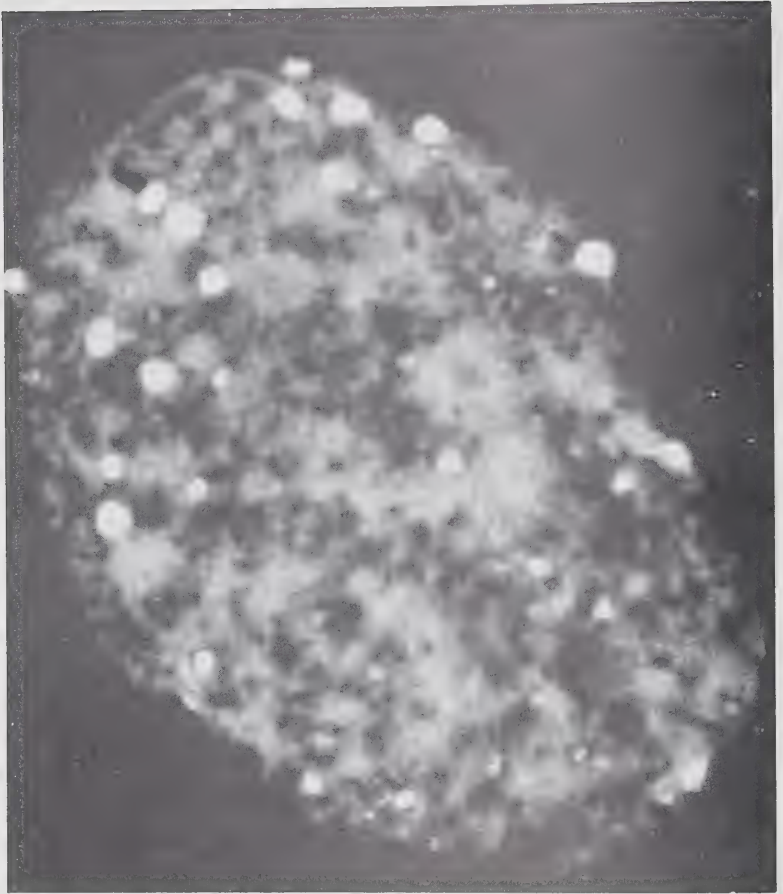


FIG. 2.
Interphase cell showing chromocentres. X1250. Compare Figure 3.

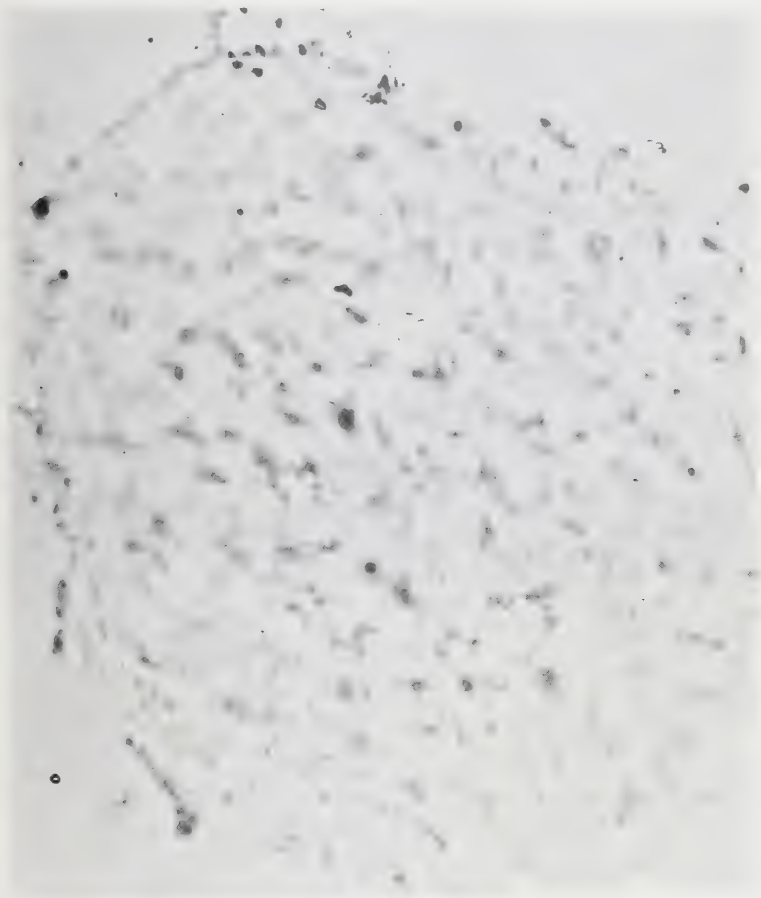


FIG. 3.

The interphase cell shown in Figure 2, photographed with phase contrast using visible light. The fields of view are identical, the nucleus appearing larger in this photograph due to rupture of the nuclear membrane having released non-fluorescent material into the cytoplasm.

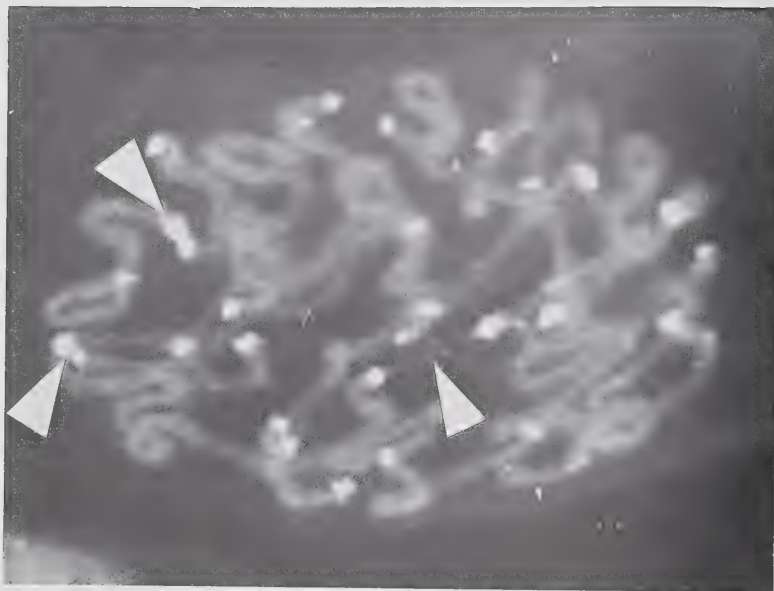


FIG. 4.

Early prophase cell, showing terminal attachments (arrowed). X1250.